PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RPS/P60638WO00	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2005/000308	International filing date *(day/month/year)* 28.01.2005	Priority date *(day/month/year)* 28.01.2004
International Patent Classification (IPC) or national classification and IPC INV. G01N1/28 G01N27/447 C12Q1/68		
Applicant NORCHIP et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (Indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.11.2005	10.05.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Smith-Hewitt, L Telephone No. +49 89 2399-2995

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/000308

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-41	as originally filed
Claims, Numbers	
1-29	received on 01.02.2006 with letter of 31.01.2006
Drawings, Sheets	
1/6-6/6	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has, within the applicable time limit:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☐ neither restricted the claims nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	29
	No:	Claims	1-28
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	29
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

   see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Re: Item I

1 The amendments have basis in the original application as filed and hence fulfil the requirements of Article 34(2)(b) PCT.

2. Re: Item VIII

2.1 It is not apparent from claim 1 whether all the component features of the subject matter should be formed on a common substrate. The term "integrated" is interpreted to mean that embodiments also where only some components are formed on a common substrate, ie where only part of the diagnostic apparatus is integrated on the lab on a chip are to be read as falling under the scope of the claims. This interpretation is reinforced in the description on page 15: The device or system *may be* microfabricated on a common substrate material, ie this is an optional feature. Similarly, p.8, 3rd paragraph, states that only "some of the components of the system" need to be microfabricated and integrated on a common substrate. Said claim is thus not clear (Article 6 PCT).

2.2 Although the applicants have indicated in their letter dated 31.01.2006 that the pump or syringe coupled to the inlet may be used to dispense all the fluids, the present wording of the claim does not actually require this. In fact the essential feature described on p.29, l.17-19, namely a single (syringe) pump for actuation of all liquids, is not incorporated in the claim (Article 6 PCT).

2.3 The term "single pumping system" may be read to mean a system with a single pump or a system with several pumps. In a single system with several pumps, the valves mentioned in claim 1 would indeed be optional, as in fact each pump would control a specific flow path. Similarly, in a single system with one pump and no valves, several inlets (as in D1, [0067]) may be used to drive individual fluids. Nevertheless, if just a single pump were used to drive all liquids (as the applicant appears to indicate is where the invention may lie), the valves mentioned as being "optional" would actually be necessary to ensure the desired flowpaths through the device. This opinion is reinforced by the two examples of single pump systems in the application (bridging paragraph on p.27-28; p.29, l.16-p.32, l.14). The requirements of Article 6 PCT are not met.

2.4 "Fluid communication" simply means that a fluid (even gas) may flow between two points.

3. Re: Item IV, V

3.1 Those features described as being "optional" in the claims are taken to be such and thus are not considered scope limiting.

3.2 Although in the disclosure of D1 the integrated system comprises a diagnostic chip integrated onto a cartridge, the wording of the present claim 1 does not exclude this possibility (see above).

The system of D1 further comprises (see in particular Fig. 2):

(a) an inlet for a fluid sample (103);
(b) a lysis unit for lysis of cells and/or particles contained in the fluid sample (107, 119);
(c) a nucleic acid extraction unit for extraction of nucleic acids from the cells and/or particles contained in the fluid sample (122);
(d) a reservoir containing a lysis fluid(109);
(e) a reservoir containing an eluent for removing nucleic acids collected in the nucleic acid extraction unit which is in fluid communication with the inlet (via the sample flow path);

wherein the sample inlet is in fluid communication with the lysis unit,
wherein the lysis unit is in fluid communication with the nucleic acid extraction unit, a valve being present to control the flow of fluid therebetween;
wherein the reservoir containing the lysis fluid is in fluid communication with the lysis unit, and wherein the reservoir containing the eluent is in fluid communication with the nucleic acid extraction unit, a valve being present to control the flow of fluid therebetween.

Furthermore, the sample input is forced to flow into the device using a pump (see [0068]). A "single pumping system" may be provided: (see [0065]; electrolytic pump located inside the cartridge, or especially [0067]; "The motive source could also be a compressor...in the instances in which an external pressure motive source is used,

the cartridge has suitable ports for interfacing with said source").

3.3 The disclosure of D1 may therefore be read onto the scope of the amended claim 1. The amended claim 1 does not meet the requirements for novelty in accordance with Article 33(1)(2) PCT.

3.4 Furthermore, the additional features of dependent claims 2-4, 6 and 10-14 are anticipated by the disclosure between paragraphs [0068] and [0205].

3.5 The disclosure of paragraph [0074], teaches that an air gap separating elution fluid and wash fluid is advantageous. As for example present claim 11 states that "the common reservoir comprises a conduit in fluid communication with the inlet and lysis unit", the additional subject matter of claims 7-9 appears to be anticipated by the arrangement of the feeding conduit to device (122). Claims 7-9 cannot therefore be considered to be novel in the sense of Article 33(2) PCT.

3.6 The subject matter of claims 16([0114]), 17-19 ([0133]-[0139]) and 20-28 [0068]-[0205] is also anticipated by the disclosure of D1.

3.7 The additional features of claim 29 are considered to be novel over the disclosure of D1. Nevertheless, in the light of the disclosure of [0074], the method of separating process fluids by air in the same chamber would appear to be trivial, especially in the light of the disclosure of paragraph [0074], which specifically teaches that "air gaps" between fluids are advantageous. Said claim is thus not considered to fulfil the requirements for inventive step under Article 33(3) PCT.

3.8 The methods described by any of claims 23-29 do not comprise either the features of or the concept of the single pumping system required by claim 1 and therefore do not fulfil the requirements of unity (Rule 13 PCT).

3.8 Document US6544734B1 (D2); (col.1, l.39 - col.2, l.47 and col.3, l.3-col.6, l.8, Abstract and Figures 1, 3) also anticipates the subject matter of claims 1-6,14-16, 19-22, 23-27. This is based on the interpretation of the term "reservoir" (claim 1) to mean any storage device for the various fluids, again due to the broad definition "integrated

lab-on-a-chip diagnostic system", not necessarily being a part of the chip per se.

3.9 Document D3 (US2003/0138941 A1, Fig. 1 and 11A, 11B; paragraphs [0082]-[0087], [0095]-[0102], [0120]-[0121], [0149]-[0153], [0158]-[0198]) also anticipates the subject matter of claims 1-6, 10-14 and 19-27. In addition, silica beads/particles are well known in nucleic acid purifying processes (see DE 197 00 364 A1, p.2, l.25-57), thus the skilled person would find it obvious to incorporate them as part of the sample preparation steps on the chip described by D3. Claims 16 and 17 are thus not considered to be inventive over a combination of the disclosures D3 and D4 (Article 33(3) PCT).

3.10 Document D5 (JOON-HO KIM ET AL, "A disposable DNA sample preparation microfluidic chip for nucleic acid probe assay", PROCEEDINGS OF THE IEEE 15TH. ANNUAL INTERNATIONAL CONFERENCE ON MICROELECTRO MECHANICAL SYSTEMS. MEMS 2002. LAS VEGAS, NV, JAN. 20 - 24, 2002, IEEE INTERNATIONAL MICRO ELECTRO MECHANICAL SYSTEMS CONFERENCE, NEW YORK, NY : IEEE, US, the whole document, especially p.133, col.2, l.2-14 and Fig.1, but more importantly Fig. 11, "Sample loading") also discloses the containment of elution solution and wash solution in the same tube separated by air gaps as a method of operating a DNA chip, and thus anticipates the subject matter of present claims 7-9. The additional features of claim 29 seem to be trivial in the light of D5.

3.11 It can also be argued that document D6 (WO00/62931, Figs., p.7, l.10-p.16, l.22 and p.36, l.28-p.40, l.10) represents very close state of the art. Said disclosure anticipates the subject matter of claims 1-6, 10-14 and 19-28. D6 even describes on chip storage chambers for lysis and eluent fluids.

4. Re: Item VI

The applicants are made aware of document D7 (WO2004/096443 A1), having a priority earlier than the present application (priority of 25-04-2003, filed on 19-04-2004) but published after (on 11-11-2004). Said document could be relevant to novelty in the regional phase.

4. Re: Item VII

The examples on p. 35-41 do not appear to fall under the scope of the claims.

CLAIMS:

1. An integrated lab-on-a-chip diagnostic system for carrying out a sample preparation process on a fluid sample containing cells and/or particles, the system comprising:

(a) an inlet for a fluid sample;

(b) a lysis unit for lysis of cells and/or particles contained in the fluid sample;

(c) a nucleic acid extraction unit for extraction of nucleic acids from the cells and/or particles contained in the fluid sample;

(d) a reservoir containing a lysis fluid in fluid communication with the inlet, an optional valve being present to control the flow of fluid therebetween;

(e) a reservoir containing an eluent for removing nucleic acids collected in the nucleic acid extraction unit, said reservoir being in fluid communication with the inlet, an optional valve being present to control the flow of fluid therebetween;

wherein the sample inlet is in fluid communication with the lysis unit, an optional valve being present to control the flow of fluid therebetween;

wherein the lysis unit is in fluid communication with the nucleic acid extraction unit, an optional valve being present to control the flow of fluid therebetween;

wherein the reservoir containing the lysis fluid is in fluid communication with the lysis unit, an optional valve being present to control the flow of fluid therebetween; and

wherein the reservoir containing the eluent is in fluid communication with the nucleic acid extraction unit, an optional valve being present to control the flow of fluid therebetween, and wherein said system further comprises a

pump or syringe for introducing a fluid sample and/or air into the inlet, whereby the system is driven by a single pumping system.

2. A system as claimed in claim 1, further comprising (g) a nucleic acid reaction unit, preferably a nucleic acid sequence amplification and detection unit, wherein the nucleic acid extraction unit is in fluid communication with the nucleic acid reaction unit, an optional valve being present to control the flow of fluid therebetween.

3. A system as claimed in claim 1 or 2, further comprising (h) a waste unit, wherein the waste unit is in fluid communication with the lysis unit, an optional valve being present to control the flow of fluid therebetween.

4. A system as claimed in any one of claims 1 to 3, further comprising (i) a reservoir containing a washing solvent, preferably ethanol, which reservoir is in fluid communication with the nucleic acid extraction unit, an optional valve being present to control the flow of fluid therebetween.

5. A system as claimed in any one of claims 1 to 4, further comprising (j) a reservoir containing a further washing solvent, preferably isopropanol, which reservoir is in fluid communication with the nucleic acid extraction unit, an optional valve being present to control the flow of fluid therebetween.

6. A system as claimed in claim 4 or claim 5, wherein the reservoir containing the eluent is in fluid communication

with the reservoir containing the first washing solvent and/or the reservoir containing the second washing solvent.

7. A system as claimed in claim 6, wherein the eluent, the first washing solvent and/or the second washing solvent are contained in a common reservoir.

8. A system as claimed in claim 7, wherein the eluent, the first washing solvent and/or the second washing solvent are separated from one another in the common reservoir by a fluid, preferably air.

9. A system as claimed in claim 7 or claim 8, wherein the common reservoir comprises a conduit in fluid communication with the inlet and the lysis unit.

10. A system as claimed in any one of claims 1 to 9, further comprising a filtration unit, which unit is in fluid communication with the lysis unit.

11. A system as claimed in claim 10, wherein the filtration unit comprises one or more of a dead-end filter, a cross-flow filter (eg micro-structured channels, porous hollow fibres or membranes), a gravity settler, a centrifuge, an acoustic cell filter, an optical trap, dielectrophoresis (DEP), electrophoresis, flow cytometry and adsorption based methods.

12. A system as claimed in any one of claims 1 to 14, wherein the lysis unit further comprises means to filter the fluid sample.

13. A system as claimed in claim 12, wherein said means comprises one or more of a dead-end filter, a cross-flow filter (eg micro-structured channels, porous hollow fibres or membranes), a gravity settler, a centrifuge, an acoustic cell filter, an optical trap, dielectrophoresis (DEP), electrophoresis, flow cytometry and adsorption based methods.

14. A system as claimed in any one of the preceding claims, wherein the system further comprises means for heating the contents of the lysis unit and/or the nucleic acid extraction unit.

15. A system as claimed in claim 14, wherein said mean comprises one or more Peltier elements located in or adjacent the lysis unit and/or the nucleic acid extraction unit.

16. A system as claimed in any one of the preceding claims, wherein the nucleic acid extraction unit is at least partially filled with silica beads or particles.

17. A system as claimed in claim 16, wherein the nucleic acid extraction unit further comprises one or more sets of electrodes adjacent the silica beads or particles for collecting and/or preconcentrating the eluted nucleic acids.

18. A system as claimed in claim 17, wherein said one or more sets of electrodes comprises platinum electrodes.

19. A system as claimed in any one of the preceding claims for extracting nucleic acids present in a biological fluid, a dairy product, an environmental fluid or drinking water.

20. An apparatus for the analysis of biological and/or environmental samples, the apparatus comprising a system as defined in any one of the preceding claims.

21. An assay kit for the analysis of biological and/or environmental samples, the kit comprising a system as defined in an one of the claims 1 to 19 and means for contacting the sample with the system.

22. An apparatus as claimed in claim 20 or an assay kit as claimed in claim 21 which is disposable.

23. A method for the manufacture of an integrated lab-on-a-chip diagnostic system as defined in any one claims 1 to 19, which method comprises:

A. providing a substrate having an inlet recess, a lysis unit recess, a nucleic acid extraction unit recess, a lysis fluid reservoir recess and an eluent reservoir recess in a surface thereof;

B. providing a cover; and

C. bonding the cover to the substrate to create the (a) inlet, (b) the lysis unit, (c) the nucleic acid extraction unit, (d) the lysis fluid reservoir and (e) the eluent reservoir, each being defined by the respective recess in said surface of the substrate and the adjacent surface of the cover.

24. A method as claimed in claim 23, further comprising the step of introducing lysis fluid into the lysis fluid reservoir either before or after bonding the cover to the substrate.

25. A method as claimed in claim 23 or claim 24, further comprising the step of introducing eluent into the eluent reservoir either before or after bonding the cover to the substrate.

26. A method as claimed in any one of claims 23 to 25, further comprising the step of introducing a first washing solvent, preferably ethanol, into the eluent reservoir either before or after bonding the cover to the substrate.

27. A method as claimed in any one of claims 23 to 26, further comprising the step of introducing a second washing solvent, preferably isopropanol, into the eluent reservoir either before or after bonding the cover to the substrate.

28. A method as claimed in any one of claims 23 to 27, wherein the eluent, and/or the first washing solvent and/or the second washing solvent are separated from one another by a fluid, preferably air.

29. A method as claimed in claim 23 or claim 24, further comprising:

introducing eluent into the eluent reservoir after bonding the cover to the substrate;

introducing a first volume of an immiscible fluid, preferably air, into the eluent reservoir;

introducing a first washing solvent, preferably ethanol, into the eluent reservoir, whereby the first washing solvent is separated from the eluent by said first volume of immiscible fluid;

introducing a second volume of immiscible fluid, preferably air, into the eluent reservoir; and

introducing a second washing solvent, preferably isopropanol, into the eluent reservoir, whereby the second washing solvent is separated from the first washing solvent by said second volume of immiscible fluid.